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EFFECTS OF MICROBIAL-PHYTOREMEDIATION ON PLANT DIVERSITY AND SOIL PHYSICO-CHEMICAL PROPERTIES IN NORTHERN SHAANXI COAL MINING AREA, CHINA

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Abstract

Effects of different microorganisms-plant combination on the diversity of plant and soil physics in the mining area were studied. Results showed that the number of species, total number and coverage of Soil Remediation + Vetiver + Sea-buckthorn (AH) treatment were 13, 298 and 89.8%, respectively, which were significantly higher than other treatments. Soil water content in different treatments was the highest at 20 cm. The pH value of the different treatments decreased significantly, and the pH value of AH was the lowest (7.14). The total nitrogen (TN) content of GH and GS were the highest, which were 0.54 and 0.51 g/kg, respectively. The contents of phosphorus (P) and available potassium (K) were the highest in AX treatment, reaching 57.1 and 194 mg/kg. Treatment with different inoculants significantly increased soil enzyme activity. The urease activity of GH was the highest, which was 0.872 mg/g. The sucrase activity and catalase content of AH and AX were the highest, which were 8.99, 8.53 mg/g and 2.145 ml/g, 1.872 ml/g, respectively. The phosphatase activities of QH and AH treatments were the highest at 234 and 229 mg/g, which were 6 times and 5.9 times than that of CK. Comprehensive analysis showed that microbial inoculum + mixed seeding mode can effectively improve soil fertility and enzyme activity, and the effect of AH was the best.

Introduction

At present, China's coal reserves are about 9.5×1012 t, accounting for 11% of the world's total coal reserves (Cui 2018), and the annual output reaches more than 1.3 billion tons. The amount of coal mining in China continues to increase, and mining cause's serious damage to vegetation, soil and local ecosystems (Liu *et al*. 2016, Hu *et al*. 2017). In response to some problems caused by coal mining, related work was carried out on ecological restoration of land in mining areas, but the current ecological restoration technology is still significantly lower than that of developed countries like Europe and the United States. In particular, the contradiction between the rich coal resources in northern Shaanxi and the fragile ecological environment has become more and more prominent in the development of resources in recent years. The exploitation of coal resources has caused a series of local geological and ecological problems (Zhao *et al*. 2016) in recent years

In view of the problems of long-term coal mining, such as vegetation destruction, soil water and fertilizer shortage, soil heavy metal pollution, ecosystem damage, etc., it is necessary to carry out much coal mine restoration technology research. Zhang *et al*. (2017) studied the effects of different plant combinations on soil ecological restoration in rare earth mines. Chang (2013)

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pointed out that the application of inoculants can improve soil nutrients and mature the soil. Zhang *et al*. (2017) has shown that the inoculation of *Fusarium* in alfalfa can effectively decompose the high-cyclic aromatic hydrocarbons in the mining area, and the highest degradation efficiency is about 15%. Bai *et al.* (2020) have shown that Sedum with ore can effectively remove heavy metal Cd in river fluvo-aquic soil and red-yellow mud, and its restoration efficiency is 26.81 and 20.79%, respectively. The average values of TN and P in the cm layer were 4.64 and 22.44 times that of recent planting, respectively. Herbs and shrubs cooperate with each other to maximize the use of horizontal space and vertical space, so that the built community presents aboveground stratification and horizontal mosaicism similar to natural communities, reducing the rate of water infiltration and improving the ability to retain water and fertilizer (Lu 2017, Xu *et al*. 2020). At present, in the process of ecological restoration in mining areas, most of them use a single plant or microbial restoration technology, and there are relatively few studies on the combined restoration of the two technologies at the same time. Thus, the present study selected *hrysopogon zizanioides* (vetiver grass) and *Hippophae rhamnoides* (sea-buckthorn) microorganisms *Rhizobia*, EM inoculants, *Bacillus* subtilis as the research objects for investigates the effects of different combination modes on soil fertility improvement in northern Shaanxi coal mining areas.

Materials and Methods

Microbial inoculants used in this study were *Rhizobium* (Zhoutian Biotechnology Company, Qihe County, Shandong), Bacillus subtilis (Shouguang Shimet Fertilizer Company, Shandong Province), EM bacteria (Shouguang Shimet Fertilizer Company, Shandong Province).

The study side was selected in Sangshuta coal mining area in the northern part of Gaojiabao Town, Shenmu City, northern Shaanxi. The soil pH was 8.81. The electrical conductivity was 10.41 mS/m. The organic matter and total nitrogen were 3.20 and 0.15 g/kg, respectively (Wang *et al*. 2021). Available phosphorus and potassium were 24.4 and 93 mg/kg, respectively. The field trials were held in March 2021. The area of a single cell was 4 × 4 m. The trials were arranged in random block design according to Table 1. The microbial inoculum was applied by the randomized block design, and the application rate of 50 kg/mu. Vetiver grass was planted at a spacing of 40 cm × 40 cm and sea-buckthorn planted at a spacing of 40 cm × 50 cm.

Table 1. Field trial settings.

Main treatment	Vetiver (X)	Sea Buckthorn (S)	Vetiver + Sea Buckthorn (H)
Side treatment	Side treatment Rhizobia (G)	Side treatment Rhizobia (G)	Side treatment Rhizobia (G)
	Bacillus subtilis (Q)	Bacillus subtilis (Q)	Bacillus subtilis (Q)
	Soil Remediation Agent (A)	Soil Remediation Agent (A)	Soil Remediation Agent (A)

Blank group without processing (CK)

Plant height was measured directly with a ruler. Biomass was determined by gravimetric method. Plant community survey was studied in October 2021. The sample plots with different treatments were surveyed to count plant species, quantity, abundance and coverage. Sample was collected from the Study area for identification.

Physical and chemical properties of soil were also recorded. Soil water content was determined by drying weighing method (Meng *et al.* 2020) and pH was measured by potentiometric method (water-soil ratio 2.5:1) (Zhang 2014). Soil organic matter was obtained by potassium dichromate-external heating method (Zhang 2014). Total nitrogen was determined by the Kelvin method. Molybdenum-antimony-scandium colorimetry was used for effective phosphorus. Available potassium was determined by flame photometry (Zhu *et al.* 2022).

Soil enzyme like urease was determined by the sodium phenate-sodium hypochlorite colorimetric method. Catalase was titrated with potassium permanganate. Phosphatase was determined by the p-nitrophenol colorimetric method. The sucrase was determined using the 3,5-dinitrosalicylic acid colorimetric method (Tao *et al.* 2022, Zhu *et al.* 2022).

Results and Discussion

Plant species in various sites is presented in Table 2. By investigating the composition of different plant species, it was found that there were 17 plant species in the restoration area, belonging to 8 families and 12 genera (Table 3). The whole area is dominated by herbs, and many species are only seen in a single plant in the plot. The combined planting plot has lush plants, rich species, and high species abundance. Compared with the control, the vegetation coverage of each planting plot increased significantly, and the vegetation coverage of the single planting herb vetiver grass and the combined planting plot was higher, especially the vegetation coverage of the combined planting plot was as high as 69 times that of the control. The root grass plot is also the dominant species. Among different inoculants, EM bacteria had the best effect on vegetation restoration.

Table 2. Survey results of plant diversity.

Treatment	Family	Genera	Species	Total	Coverage (%)	Number of dominant species
CK	1	1	2	21	1.3	7
AX	4	7	11	245	82.4	200
AS	2	4	7	97	43.4	32
AH	**5**	**10**	**13**	**298**	**89.8**	**150**
QX	2	8	9	201	74.7	160
QS	2	4	7	145	37.6	28
QH	3	5	9	224	82.8	120
GX	2	3	6	187	71.5	144
GS	2	5	6	131	36.8	16
GH	3	4	7	214	78.9	100

CK=Blank group without processing; AX=Soil Remediation Agent + Vetiver; AS=Soil Remediation Agent + Sea Buckthorn; AH=Soil Remediation Agent + Vetiver + Sea Buckthorn; QX=Bacillus subtilis+ Vetiver; QS=Bacillus subtilis + Sea Buckthorn; QH=Bacillus subtilis + Vetiver + Sea Buckthorn; GX=Side treatment Rhizobia+ Vetiver; GS=Side treatment Rhizobia + Sea Buckthorn; GH=Side treatment Rhizobia + Vetiver + Sea Buckthorn.

The soil water content of sea-buckthorn and mixed seed treatments increased gradually in the soil layer of 0-40 cm; while the soil water content of vetiver planting showed a decreasing-increasing-decreasing trend, with the highest values appearing at 0-20 cm. It showed that after planting sea-buckthorn, root water absorption occurred in deeper soil; herb roots mainly grew at 30-50 cm, resulting in lower water content than other soil layers. The AH treatment can increase soil water content and meet the needs of plant growth. This is conducive to improving restoration efficiency of the mining area (Fig. 1).

According to the Chinese soil pH classification standard, the soil of the coal mine area is a strong alkaline soil. The pH of different treatment has dropped down 1.01-1.51, indicating that the combined repair of the microbioplasses-plant combined repair can improve the pH of the soil in the coal mine area. Soil Remediation + Vetiver + sea-buckthorn (AH) treatment has the most obvious regulatory effect on soil pH, its pH value is the lowest (7.14); followed by AX and QX. It shows that soil repair agents and herbs have a significant improvement in the pH of the soil (Fig. 2).

Table 3. Plant species, family, and genera.

No.	Species	Family	Genera
1	*Lotus corniculatus* L	Fabaceae	Medicago
2	*Anemarrhena asphodeloides* Bunge	Liliaceae	Anemarrhena Bunge
3	*Hemerocallis citrina* Baroni		Hemerocallis L.
4	*Hemerocallis fulva* (L.) L.		Hemerocallis L.
5	*Artemisia capillaris* Thunb.	Asteraceae	Artemisia L.
6	*A. sacrorum* Ledeb. var.		Artemisia L.
7	*A. annua* Linn.		Artemisia L.
8	*Ixeris polycephala* Cass.		Ixeris Cass.
9	*Taraxacum mongolicum* Hand. -Mazz.		Taraxacum F. H. Wigg.
10	*Platycodon grandiflorus* (Jacq.) A. DC.	Campanulaceae	Platycodon A. DC.
11	*Orghum 'Bicolor'* (L.) Moench	Poaceae	Sorghum Moench
12	*Sorghum halepense* (Linn.) Pers		Sorghum Moench
13	*Cynodon dactylon* (L.) Pers.		Cynodon Rich.
14	*Ulmus pumila* L.	Ulmaceae	Ulmus L.
15	*Populus simonii* Carr	Salicaceae	Populus
16	*P. euphratica* Oliv.		Populus
17	*Potentilla discolor* Bge.	Rosaceae	Potentilla L.

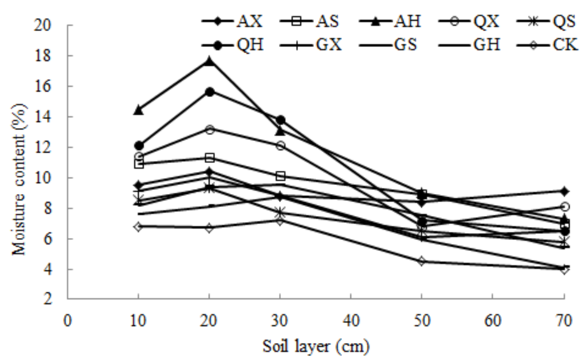

Fig. 1. Effects of different microbial agents.

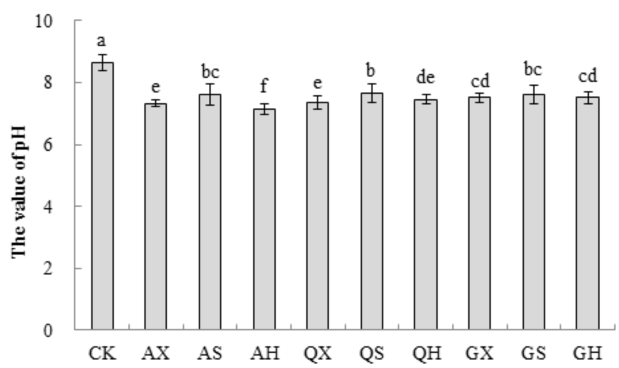

Fig. 2. Effects of different microbial on the moisture content of the soil agents on the pH of the soil.

The soil nutrient content in the coal mining area with different treatments increased to a certain extent (Fig. 3). The soil organic matter content in the AX, GX and QX treatments was significantly higher than that in the control group, increasing by 25.7, 24.8 and 23.9%, respectively, while other treatments had no significant difference. The soil organic matter content under different microorganism + vetiver treatments was significantly higher than that of sea-buckthorn and mixed planting treatments, indicating that microorganism + vetiver had a significant synergistic effect on the improvement of soil organic matter content. The increasing effect of different inoculants on TN showed the order of *Rhizobium* > EM bacteria >*Bacillus subtilis*. Among the different plant treatments, the effect of sea-buckthorn on the increase of TN was significantly higher than that of vetiver. GH and GS has the highest TN content, which are 0.54 and 0.51 g/kg. It showed that rhizobia can effectively infect the root system of sea-buckthorn and improve the nitrogen fixation ability of sea-buckthorn; while the infecting ability of rhizobia to vetiver was weak, and it cannot create a mutually beneficial and symbiotic environment. The P and K content in AX were found to reach 57.1 and 194 mg/kg, which are respectively 139.9 and 104.2% higher than CK. This is followed by AH and QX treatments, which were 55.6, 51.2 and 178, 174 mg/kg, respectively.

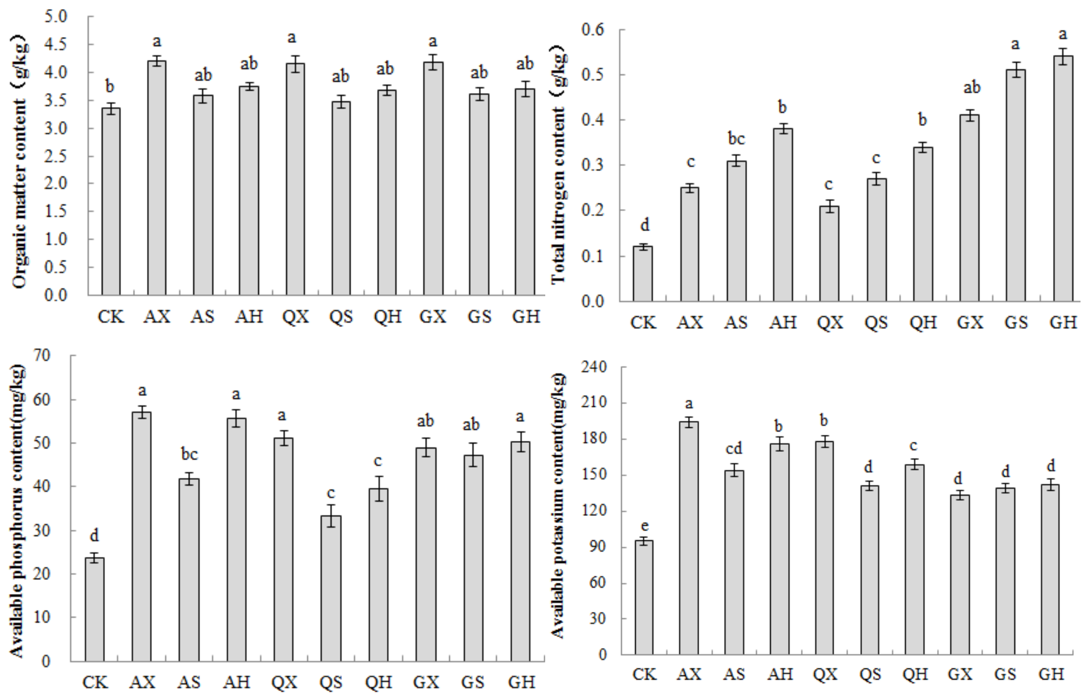

Fig. 3. Effects of different microbial agents on contents of available soil organic matter, TN, P and K in coal mining area.

By analyzing soil nutrient, it was found that the effect of herbs was better than that of shrubs. The possible reason might be that the planting years were short, the herbs grew rapidly, and the nutrient exchange with the soil was completed quickly to achieve a balanced symbiosis. *Rhizobium* can significantly increase nitrogen content in different inoculants, but the comprehensive effect of EM bacteria is the best. According to the change of the overall nutrient level in the soil, AX and AH treatments had the best effect on soil nutrients.

Table 4. Effects of different treatments on soil enzyme activities.

Treatment	Urease (NH_3-N)/mg·g^{-1}	Sucrase (glucose)/ mg·g^{-1}	Phosphatase (phenol)/ mg·g^{-1}	Catalase (0.1NKMnO$_4$)/ ml·g^{-1}
CK	0.409 d	4.9 e	39 f	0.341 g
AX	0.545 c	8.53 ab	203 c	1.872 a
AS	0.512 c	7.95 b	181 d	1.546 b
AH	0.623 bc	8.99 a	229 ab	2.145 a
QX	0.523 c	8.02 b	218 bc	1.087 c
QS	0.508 c	6.76 c	198 cd	0.726 e
QH	0.593 bc	8.43 ab	234 a	1.354 b
GX	0.674 b	7.63 b	175 de	0.854 d
GS	0.609 bc	5.61 d	168 e	0.608 f
GH	0.872 a	8.06 b	201 c	1.104 c

Table 4 showed that the different treatments significantly improved the enzyme activities in soil. The urease activity in the soil increased with the addition of microbial inoculants. The urease activity in the soil treated with the rhizobia inoculum was significantly higher than that in the other treatments. From the perspective of different planting patterns, the urease activity in the soil under the shrub-herb hybrid treatment was significantly higher than that of the single species. From the planting type, the soil urease activity of sea-buckthorn was higher than that of vetiver grass plots. The urease activity of GH was the highest (0.872 mg·g^{-1}), which was 113.2% higher than that of CK. Different microbial bacteria-mixed species significantly increases the invertase activity in soil. The order of invertase activity from high to low was in the order AX > AH > QH > GH > QX > AS > GX > QS > GS > CK. The sucrase activities of AH and AX were the highest (8.99 and 8.53 mg·g^{-1}), which were increased by 83.5 and 74.1% compared to CK, and the differences between them were significant ($P < 0.05$). The soil alkaline phosphatase activity was significantly enhanced after microbial inoculum-plant combined remediation. Among them, the phosphatase activities treated with QH and AH were the highest at 234 and 229 mg·g^{-1}, which were 6 times higher than that of CK. The change trend of soil catalase activity in different treatments was basically consistent with the change trend of sucrase activity. The catalase contents of AH and AX were 2.145 and 1.872 ml·g^{-1}, respectively, which were 6.3% in CK.

By planting different plants, the plant species and coverage in the mining area can be effectively improved, which is consistent with the research results of Yan DZ (2007) and Lu *et al*. (2017). The application of microbial bacterial fertilizer can increase the content of organic matter in the soil, improve the living environment of microorganisms, provide nutrients for microorganisms continuously, and promote their growth. This is consistent with existing research results (Quan *et al*. 2016, Mao *et al*. 2019). Previous studies have shown that the application of fungicide is beneficial to improve soil physical and chemical properties and microbial flora, increase the number of microorganisms, and improve the activities of catalase, urease, phosphatase and invertase in the soil (Marcote *et al*. 2001, Zhang *et al*. 2021, Ezeokoli *et al*. 2019), which is consistent with the results obtained in the present study. The results are consistent. The application of three different inoculants significantly increased the soil enzyme activity. Among them, the soil remediation agent containing EM bacteria had the best effect. At the same time, this study found that the overall effect of microbial inoculants + herbs was better than that of shrubs. This is because in the early stage of the experiment, herbs grew rapidly and their metabolic activity was significantly higher than that of shrubs, so root exudates could effectively improve soil microbial activity. Herbs can grow rapidly and complete the material capacity cycle with the soil. Therefore, the present study showed that vetiver has a better effect on soil nutrient improvement. Sea-buckthorn can significantly increase the nitrogen content in soil through nitrogen fixation (Fu XR 2017).

Comprehensive analysis showed that different pioneer plants has obvious effects on vegetation restoration in mining areas. The soil water content, nutrients and enzyme activities were significantly increased in different treatments. Therefore, the microbial inoculum + hybrid mode can effectively increase the vegetation coverage and diversity in the mining area, and improve the soil environment. The repair effect of AH was the best.

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